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The therapeutic potential of targeting the HGF/cMET axis in ovarian cancer

Kim Moran-Jones

Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow, United Kingdom, G61 1QH.

The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, 2010, Australia.

E-mail: Kim.Moran-Jones@Glasgow.ac.uk

Ph: +44 (0)141 330 2830.

Running title: *Targeting HGF/cMET in ovarian cancer*

Abstract

Survival rates for ovarian cancer have remained relatively stable for the past two decades, despite advances in surgical techniques and cytotoxic chemotherapeutics, indicating a requirement for better therapies. One pathway currently proposed for targeting is the HGF/cMET pathway. Up-regulated in a number of tumour types, cMET is a tyrosine kinase receptor expressed on epithelial cells. In ovarian cancer, it has been identified as highly expressed in the four major subtypes, with expression estimates ranging from 11-68% of cases. HGF, the only known ligand for cMET, is found at high levels in both serum and ascites in women with ovarian cancer, and proposed to induce both migration and metastasis. However, clinically validated biomarkers are not yet available for either HGF or cMET, preventing a clear understanding of the true rate of over-expression, or its correlation with prognosis. Despite this, a number of agents against HGF and cMET are currently being investigated in clinical trials for multiple tumour types, including ovarian. However, a lack of patient selection, biomarker usage, and post-hoc analysis correlating response with expression, has resulted in the majority of these trials showing little beneficial effect from these agents, indicating that additional research is required to determine their usefulness in patients with ovarian cancer.

Bullet points

- In the absence of clinically validated biomarkers, the frequency of high HGF/cMET signalling is unknown.
- Therefore it is not possible to correlate prognosis
- HGF/cMET-targeted therapies are being used without knowing whom they will benefit.

1. Introduction

Ovarian cancer is the deadliest of the gynaecological malignancies, and is the 5th commonest cause of cancer-related deaths in women [1]. Survival rates for epithelial ovarian cancer (EOC), which accounts for 90% of ovarian cancer diagnoses, have remained relatively stable for the past two decades, despite advances in surgical options and chemotherapeutics.

1.1 Classification of EOC

In recent years, EOC has been classified as being of Type I or Type II, with the former being described as being indolent, and on a molecular trajectory from benign to atypical proliferative through to invasive tumours. These Type I lesions typically remain within the ovary, and are classified as genomically stable, without *TP53* mutations, and comprise the low-grade serous-papillary, endometrioid, clear cell, mucinous, tumours, and borderline tumours of low malignant potential [2]. Type II EOC, on the other hand, are thought to be *de novo* aggressive, and are frequently diagnosed when metastases are already present. They are genetically unstable, and universally contain TP53 mutations and dysfunctional breast cancer genes, *BRCA1/2,* (which results in further chromosomal disruption). Their origin has long been debated, but current evidence points to the secretory epithelium of the distal fallopian tube (reviewed in [2, 3]). High grade serous carcinomas, high grade endometrioid, and undifferentiated carcinomas [2] are classified as type II epithelial ovarian cancers. Overall survival rates for the two types differ dramatically, with median survival being 82 months [4] versus 30 months [5] for Types I and II, respectively. This difference is despite the initial sensitivity of type II tumours to standard carboplatin and paclitaxel chemotherapy. However, a recent study performed by Panici and colleagues suggest that the survival between the two groups to be far closer (72 months vs 62 for types I and II respectively [6]), indicating that there is still much we don't understand about this disease. Adding to this argument, is the finding that clear cell ovarian cancer (CCOC) is genetically separate to other forms of ovarian cancer, with different common mutations [7], and *de novo* chemo-resistance.

Ongoing molecular characterisation and genetic studies suggests that this model may be overly simplistic, with a number of subtypes being re-classified, or further divided into additional sub-groups [8, 9, 7, 10]. These new subtypes demonstrate different genetic lesions and transcriptional profiles, chemo-sensitivity, and survival rates,

indicating a need to incorporate this information into treatment plans for ovarian cancer.

1.2 Current treatment of patients with EOC

Currently, most women diagnosed with ovarian cancer, regardless of subtype or Type, undergo total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), and omentectomy surgery, to remove the ovaries, fallopian tubes, uterus and omentum. In cases of advanced ovarian cancer, debulking of all other visible tumour deposits is also performed with the aim of achieving complete macroscopic clearance of disease. Patients are usually treated with a cytotoxic chemotherapy post-surgery, invariably a platinum based regimen. For women with advanced disease (stage 3C/4) at presentation, initial treatment with chemotherapy can be undertaken prior to surgery, which can reduce surgical morbibity but does not improve overall survival [11]. Despite this invasive surgery and treatment, the majority of women recur within 2 years [12].

In recent years, as a better understanding of the molecular landscape of EOC has become available, a number of targeted therapies have been proposed and trialled.

2. HGF/cMET in the ovary

A pathway attracting attention as a potential target in ovarian cancer is the HGF/cMET axis. cMET is a receptor tyrosine kinase, typically expressed on epithelial cells, and is present in both embryonic and adult ovarian tissue [13, 14] . HGF (hepatocyte growth factor), the only known ligand for cMET, is expressed on cells of mesenchymal origin, and acts as an epithelial cell mitogen, motogen, and morphogen. During ovarian development, HGF is produced by mesenchyme within the uro-genital ridge adjacent to cMET-expressing epithelial cells, suggesting an involvement of this pathway in ovarian development and proliferation [15]. Paracrine signalling between the ligand and receptor has also been demonstrated during the development of the ovary and follicles [16-19]. In the adult ovary, the ovarian surface epithelium and granulosa cells express cMET, while the stroma and theca cells produce HGF [20]. HGF expression is also regulated by a number of ovarian hormones [21, 18], indicating a role for this signalling axis in oocyte maturation.

2.1 HGF/cMET in ovarian cancer

In recent years, mis-regulation of the HGF/cMET pathway has been investigated in ovarian cancer, and high expression of cMET has been identified in subsets of all four

major histotypes of EOC (high grade serous, clear cell, mucinous, and endometrioid [22-29]). It must be noted, however, that the outcome from this over-expression remains unclear. While several studies have demonstrated a correlation between high cMET expression and poor prognosis [25, 30], others have shown no statistically significant correlation between cMET expression and shorter survival [26, 29], and still others suggest that cMET is expressed in early tumours, and is associated with good prognostic factors [23]. It should be noted that the majority of these studies were performed on relatively small numbers of samples, and usually contained mixed subtypes of EOC, thus further confusing the matter. These findings are summarised in Table 1.

High levels of the cMET ligand, HGF, in serum in women presenting with a pelvic mass is indicative of ovarian cancer (>2 SD above serum levels in women with benign ovarian tumours), and is predictive of poor prognosis in women with advanced EOC [31]. HGF is also present at high levels in the ascites of patients with ovarian cancer [32] [33], and is proposed to induce ovarian carcinoma cell migration and metastasis. Similarly, high expression of HGF in tumour cells has been correlated to decreased progression-free survival, and higher serum CA-125 and CA19-9 levels [34]. While HGF is typically expressed in the stroma, in ovarian cancer samples, it appears to be expressed in the epithelial cells [29, 35, 19, 32], potentially generating an autocrine loop. However, it has been previously noted that HGF can regulate its own expression [36] as well as that of cMET [37], setting in place an auto-amplification loop, and it is possible that one of the early steps of ovarian carcinogenesis is the co-expression of HGF and cMET in ovarian surface epithelium. This is supported by evidence showing that cultures of ovarian surface epithelium (OSE) from women with a family history of ovarian cancer express both HGF and cMET, whereas similar cultures from women with no family history do not [38], although how this pertains to the fallopian tube origin of ovarian cancer is yet to be ascertained. This autocrine loop is most likely then perpetuated by the high expression of HGF within the ascites fluid [32, 33]. HGF present in ascites also impacts the mesothelial cells of the peritoneum, inducing a mesothelial-to-mesenchymal transition (MMT), which may provide a more favourable environment for ovarian cancer cells to colonise and invade [34]. Kenny *et al.* demonstrated that ovarian cancer cells preferentially adhered to omental fibroblasts rather than mesothelial cells [39], which may explain the increased adhesion in the presence of HGF, where MMT has been induced, and

HGF enhances the adhesion of ovarian cancer cells to mouse peritoneum [40]. Interestingly, use of a HGF-neutralising antibody in a mouse model of ovarian peritoneal metastasis resulted in decreased tumour cell dissemination and ascites formation [34], adding further weight to the logic in targeting the HGF/cMET axis in ovarian cancer. Data is currently accumulating that clear cell ovarian cancer in particular displays a high rate of cMET expression, and specific MET gene amplification [41-44]. Given this subtype's poor prognosis, chemo-resistance, and its genetic separation from other forms of ovarian cancer, subtype-specific treatment merits further investigation.

3. Mis-regulation mechanisms of HGF/cMET

cMET was originally identified as an oncogene as a result of a chromosomal rearrangement that fused the translocating promoter region (tpr) to the cMET kinase domain [45]. The same rearrangement has since been identified in precursor lesions of gastric cancer, suggesting that it can pre-dispose to the development of gastric carcinomas [46]. Unlike the majority of oncogenes, mutations within the kinase domain of cMET are not frequent, although mutations have been found outside of the kinase domain (reviewed in [47]). Amplification of the cMET gene, or polysomy (of chromosome 7) is detected in a number of cancer types, and has been correlated with poor prognosis, high protein expression, and ligand-independent activation of Met (reviewed in [47]). As yet, there is no clinically validated method for defining cMET amplification, and thus the actual rate of amplification in tumour samples varies significantly between studies. The correlation between sensitivity to cMET-inhibitory agents and cMET amplification remains unclear, with various studies reporting correlation [48-51], transient sensitivity [52], or no correlation [53-55], and as such it will be important to define a method by which copy number can be accurately determined and clinically validated. In high grade serous ovarian cancer, the TCGA cBioPortal [56, 57] reveals mutations in the Met gene to be present in 1.3% of cases, and amplifications in 1.6%. However, neither mutation nor amplification was associated with survival. High cMET expression has also been noted in the absence of mutation or amplification, most likely a result of transcriptional up-regulation. A number of factors are known to result in increases in cMET expression, including hypoxia [58], and activation of other oncogenes, such as Ras and Ret [59].

HGF is rarely found to be mutated or amplified in cancer, and a search of the TCGA cBioPortal [56, 57] reveals HGF to be mutated in 0.6% of high grade serous ovarian cancer patients (with mutations resulting in truncation), and amplified in 1.3% of cases. Although an association between the rs1800793 single nucleotide polymorphism and ovarian cancer mortality has been identified, this did not correlate with changes in protein or mRNA expression [29]. However, transcriptional up-regulation has been observed resulting from Stat-3 and c-Src expression [60], and the co-expression of HGF and cMET in tumour cells can drive autocrine activation, as well as increased transcription for both genes. A number of studies have identified that HGF expression and autocrine cMET activation decreases sensitivity to cMET inhibitors, and stromal HGF levels have been linked to clinical responses in patients treated with the anti-HGF agent, ficlatuzumab [61-63], demonstrating the importance of the use of biomarkers in predicting clinical responses.

In the absence of ligand, cMET can also be activated by integrin interaction, the hyaluronan receptor CD44, plexins, and interaction with other receptor tyrosine kinases such as EGFR, RET, & Ron kinase, and some G protein coupled receptors [64-68], indicating that it may be necessary to use cMET inhibitors in combination with other agents.

4. Therapeutic agents against HGF/cMET

A number of therapeutic agents targeting HGF or cMET have been evaluated in pre-clinical cancer models or clinical trials for various solid cancers in recent years, with varying levels of success. Broadly, these agents fall into two categories: either antibodies targeting HGF or cMET, the majority of which prevent ligand-receptor interaction; or small molecule inhibitors, typically designed to block phosphorylation of cMET, and thus prevent downstream signalling. Gene therapy using adenoviruses expressing HGF-antagonist intra-molecules is also under investigation in patients [69]. Details of agents recently in clinical trials are detailed in Table 2. It has become apparent in recent years that single targeted drug therapy is usually ineffective, and frequently results in resistance. As such, it may ultimately be necessary to combine anti-HGF/cMET therapeutics with additional agents, such as those targeting EGFR, PI3K/Akt/mTOR, or MEK/ERK pathways. However, the efficacy and target population of anti-HGF/cMET agents must be defined first.

Clinical trials were traditionally designed to test agents which were assumed to have the same effect on all individuals, most recently cytotoxic chemotherapeutics targeting generic disease mechanisms. Such trials are most efficacious when they assess a potential therapeutic effect that is about the same size or slightly smaller than the effect of the natural variation that exists between individuals [70]. One of the surprising details to come out of recent trials using HGF or cMET targeting agents is the absence of patient selection using biomarkers, or indeed correlation of results post-hoc, with HGF/Met expression.

4.1 HGF/cMET therapeutics in ovarian cancer

While few of the phase I trials have included patients with ovarian cancer (see Table 3 and below), these have been sufficiently promising to merit a phase II trial using rilotumumab in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma [71](NCT01039207). However, using primary endpoints of tumour response and six-month progression-free survival, and secondary endpoints of progression-free and overall survival, this study concluded that, although rilotumumab was well tolerated, it had limited activity, and would not be investigated further as a single agent. The authors of the study conclude that use of predictive biomarkers might guide a more targeted approach to treatment in the future, highlighting a recurring theme in the use of both cMET- and HGF- targeted agents.

5. Biomarkers for the HGF/cMET pathway

Although a number of studies have attempted to define biomarkers for over-activation of the cMET pathway, no clinically validated tests are currently available. Immunohistochemical staining for cMET and phosphorylated cMET, quantitative RT-PCR for Met and HGF, and gene amplification of Met, have all been reported in the literature as potential biomarkers for selection of patients who may benefit from HGF/cMET-targeting agents. However, the variation in reported incidences of over-expression/amplification within similar cohorts of patients confirm the need for carefully validated assays.

5.1 IHC for cMET

Estimates of the frequency of HGF/cMET dysregulation differs between tumour types, but even within individual tumour types, there exists a wide variation in the reported frequency. For example, recent IHC studies performed on gastric tumour samples have reported cMET over-expression in 4-63% of cases [72-77] and various

studies performed in ovarian cancer estimate high expression of cMET to be present in 11-68 % of cases [30, 22, 23, 26, 25] (Table 1). The majority of the estimates above come from IHC studies, using a number of different antibodies, but no consensus on scoring criteria yet exists, nor whether cytoplasmic or membranous staining for cMET is important. Work performed by Koeppen and colleagues has described the validation of the CONFIRM anti-total cMET (SP44) rabbit monoclonal antibody on formalin-fixed paraffin-embedded tissue as part of the phase II trial testing onartuzumab in non-small cell lung cancer [78]. Using cell lines with known levels of cMET expression, comparisons between SP44 antibody staining, flow cytometry (using a different anti-Met antibody), and mRNA levels, it was determined that the SP44 antibody specifically recognises cMET, and not the closely-related Ron receptor. Furthermore, a comprehensive clinical scoring system was determined, which enabled a cut-off of 50% (of cells stained moderate/strong) to be utilised in the trial to differentiate patient outcomes when treated with onartuzumab [78]. A number of additional antibodies (D1C2, and A2H2-3) have also shown promise for use in clinical studies for detection of cMET levels. However, additional validation on larger cohorts of clinically annotated samples will be required prior to their incorporation into clinical practice (Koeppen et al, ASCO 2014, abstract 11103, and [79]).

5.2 Gene amplification and copy number of Met

Other studies have used gene amplification (GA) or gene copy number (GCN) to determine if cMET has been amplified (either the gene itself, or larger portions of chromosome 7) [80, 81, 73, 78] although the usefulness of cMET genetic amplification as a biomarker, and its correlation to drug sensitivity, has yet to be validated in large scale trials. Similarly, the correlation between copy number, protein expression level, and responsiveness to cMET-inhibitory agents is still under investigation [78].

5.3 Phosphorylated cMET

In a number of cancer types, including ovarian, amplification and mutation of cMET are relatively rare, making expression or activation (phosphorylation) a better readout of activity of the receptor. Although a number of studies have incorporated staining for phosphorylated cMET, it is rare that samples both pre and post-treatment are available, and not yet clear that a reduction in IHC signal with treatment correlates to a therapeutic response in the patient. Eder and Yap and colleagues both showed decreases in phosphorylated c-Met in tumour samples after treatment, although the

best patient responses identified were stable disease [82, 83]. Adding to the complexity of this matter is the labile nature of the phospho group (and indeed many other post-translational modifications), and its preservation in formalin-fixed paraffin-embedded tissue. A number of studies have demonstrated that proteins are rapidly de-phosphorylated upon oxygen deprivation, with the majority of phosphoproteins being lost if not fixed within 60 minutes [84, 85]. Additionally, the stability of different phsopho-epitopes is proposed to vary, both within a single tumour type, and between tissue types [86] making scoring and/or quantification of phospho-antibody signals inherently difficult. All of these factors decrease the reliability of phospho-cMET as a reliable biomarker for over-activation of the pathway, making selection of patients for anti-HGF/cMET targeted therapies more difficult. It should be noted that these difficulties are not unique to the HGF/cMET pathway, but applicable to increasing numbers of therapeutics which target pathways up-regulated in cancer cells, many of which are defined by phospho-activation rather than intrinsic genetic lesions.

5.4 Circulating HGF

While circulating HGF has been reported as elevated in patients with cancer in a number of studies, its use as a biomarker is still under investigation. Both onartuzumab and ficlatuzumab, therapeutic antibodies directed against HGF, stabilise the protein, and cause increases in the serum with treatment [87, 61, 88]. This enables circulating HGF to be used as a pharmacodynamic marker for these agents, but perhaps not as a catch-all predictive biomarker for selecting patients for HGF/cMET therapies. Furthermore, elevated HGF levels are observed in a number of disease settings, including virus/bacterial infections, graft-versus-host disease, and following surgical procedures, making their use as a biomarker for selection of patients less favourable [89-91]. It is also yet to be vigorously validated how circulating HGF levels relate to HGF levels within the tumour micro-environment [92]. Because HGF is a secreted, soluble, factor, it must be noted that HGF within the tumour tissue may not have been generated locally.

5.5 A requirement for biomarkers

Confusing the biomarker discussion is the finding that two patients with alveolar soft tissue sarcoma, whose archival tumour tissue was negative for c-Met IHC, and who have been treated with tivantinib, a cMET inhibitor, for >3 years, have maintained

stable disease for that period [93]. This may indicate that additional biomarkers need to be identified, or that fresh tumour biopsies need to be acquired prior to treatment commencement, to assess the cMET status more accurately, or that cMET-independent actions of tivantinib are involved [94].

Recently, the need for biomarkers when targeting the HGF/cMET pathway has become apparent, with the finding that patients with NSCLC with low Met expression do worse when receiving onartuzumab (a monoclonal antibody targeting Met) + erlotinib than erlotinib + placebo [95]. It was proposed by Spigel and colleagues that dual inhibition of EGFR (by erlotinib) and Met might have different consequences in tumours with lower versus higher Met expression, such that this effect may only be seen on a back ground of EGFR inhibition. However, a similar finding was reported in a study utilising rilotumumab (AMG 102, an anti-HGF monoclonal antibody) in metastatic gastric or esophagogastric junction cancer (Oliner, et al., ASCO 2012, abstract 4005). It should be noted however, that the final study report for this trial [96] showed no difference in progression free- or overall- survival between Met low patients receiving rilotumumab + chemotherapy, or chemotherapy alone.

What is becoming quite apparent, is that not everyone may benefit from receiving targeted therapies such as those against HGF/cMET, and, dismayingly, some receiving the therapy may in fact do worse than those receiving placebo. This makes it imperative to determine the population who will benefit, and thus to incorporate appropriate biomarkers into all studies moving forward. For ovarian cancer, which is typically diagnosed late, and where the opportunity for 2nd line treatment is limited, it is vital that only patients who will potentially benefit from an agent receive it.

6. HGF/cMET inhibitors trials in ovarian cancer

As described above, the only study with results available addressing the use of agents targeting HGF/cMET in ovarian cancer is that in which patients with persistent or recurrent ovarian epithelial cancer, fallopian tube cancer, or primary peritoneal cancer were treated with rilotumumab (NCT01039207) [71]. This trial was stopped in the first phase of recruitment, due to a lack of sufficient positive results to progress to the second phase of the trial. Of the 31 patients treated, 1 achieved a complete response, and six experienced stable disease (all patients had previously received platinum-

based therapy). However, surprisingly, no biomarkers were used, nor were analyses performed post-hoc on patients responding to treatment. With current estimates of high cMET expression occurring in 11-68% of ovarian cancer cases (see Table 1) [30, 22, 23, 26, 25], it is possible that as few as 3 patients enrolled within the study were in a position to benefit from receiving the anti-HGF antibody, and that as many as 29 patients potentially to do worse upon receiving the therapy.

Interestingly, a number of phase I trials of HGF/cMET targeting agents have included patients with ovarian cancer, [82, 97, 88, 54, 98, 61] several of which have reported favourable responses, with some correlated to biomarkers. Preliminary data presented by Buckanovich and colleagues (Buckanovich et al, ASCO 2011, abstract 5008) described a phase II discontinuation trial which had been halted due to high clinical activity seen in women with ovarian cancer treated with cabozantinib, a dual cMET and VEGFR2 inhibitor, although these data are yet to be published. However, there are ongoing trials with cabozantinib directed at women with recurrent, progressive, or persistent, epithelial ovarian, fallopian tube, or peritoneal cancer (NCT02315430 and NCT01716715).

6.1 Biomarker-based clinical trials

With relatively small numbers of individuals likely to benefit from targeted therapies, such as those against the HGF/cMET pathway, it may prove more efficacious to utilise biomarker-based trials (rather than trials based on the primary site of a tumour), such as the recent NCI Molecular Analysis for Therapy Choice (MATCH) trial, in which more than 200 actionable mutations/amplifications/translocations were assessed, and patients were matched to investigational drugs directed to the lesion of interest that their tumour harboured. Although randomised clinical trials remain the gold standard for assessing the efficacy of new agents, their design is not feasible/optimal time- or expense- wise for patients with rapidly advancing malignancies, or for agents designed against mutations present in a small proportion of the population.

7. Conclusions and recommendations

There is an urgent need to identify new therapeutics for patients with ovarian cancer, and the HGF/cMET signalling pathway merits further investigation. However, there are a number of factors that need to be addressed in order to assess the utility and efficacy of such therapeutics. First and foremost is the requirement for good

biomarker/s that can be used clinically, such that the true percentage of patients with each subtype of EOC exhibiting high HGF or cMET activity can be assessed. This will require the use of large sample sizes, and agreement as to how to score such samples. However, in recent years, IHC assays to determine HER2 status in various cancers have been successfully implemented clinically, and are now used as determinants for treatments in breast and gastric cancer. It seems likely that IHC will be used, as amplifications and mutations are relatively rare, and to date, little correlation has been seen between amplification, protein expression, and sensitivity to inhibitors. Using a validated system to measure cMET will also enable retrospective analyses as to the prognostic value of expression. Of grave importance is determining the consequences of treating individuals with low cMET expression with cMET inhibitors, and again, this will require the incorporation of biomarkers into ongoing trials, and should be a requirement for enrolment in the first instance.

Small molecule inhibitors and therapeutic antibodies have the potential to change treatment options and survival rates in many cancers. However, these agents are designed to target particular genetic lesions or specific aberrations in signalling pathways, and thus it is logical, and likely to only be of benefit to, individuals harbouring the lesions/signalling aberrations against which the agents are designed. In order to accomplish this, it is imperative that we utilise biomarkers to both identify such patients, and to be able to determine the usefulness of such agents in these patients.

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Compliance with Ethical Standards

Conflict of Interest

The author, KMJ, declares no conflicts of interest

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Biomarker examined	Samples tested	*n*	Method used	Definition of positive	% positive	Conclusion	Reference
cMET expression	Stage IIIC primary tumours	41	IHC – no quantification of intensity	>20% of cells stain positive	60.9	cMET expression associated with higher grade tumours, more para-aortic lymph node involvement, and worse overall survival.	[30]
cMET over-expression	EOC patients	156	IHC – intensity scale 0-3	Intensity of 3	27.2	cMET over-expression was associated with an advanced tumour stage.	[22]
cMET expression	Primary carcinoma	67	Southern and western blotting	N/A	28% over-expression by western, no amplification by Southern.	Expression was higher in lower-grade tumours. No correlation was identified between cMET expression and overall survival.	[23]
cMET expression and mutation status	Ovarian carcinomas	119	IHC – intensity x cellularity	Score >6	68	cMET over-expression not correlated with different histologic subtypes, grades, nor tumour stages, indicating that over-expression may be an early event.	[26]
cMET over-expression	Advanced stage ovarian cancers	138	IHC – intensity scale 0-3	Intensity of 3	11	cMET over-expression correlated with poorer median survival.	[25]
Malignant							

	n tumours and OSE material						
cMET, P-cMET, and HGF expression, and Met mutation analysis	Ovarian Cancer (adenocarcinoma/small cell/endometrioid)	40 (36/1/3)	IHC - intensity scale 0-3	Intensity 1-3	37% positive for HGF staining, 30% positive for cMET expression, 33% positive for P-cMET. No mutations found.	N/A	[27]
cMET expression	Primary ovarian carcinoma	79	IHC - intensity x cellularity, both 0-3	Any signal	92	No apparent association of cMET expression with stage, response to primary platinum therapy or CA125 levels.	[28]
HGF SNPs, HGF, cMET and P-cMET expression	Various	Varying depending on study	IHC – intensity x cellularity, and analysis of TCGA data	IHC – moderate-strong in >10% of cells.	Moderate-strong staining: HGF – 83.3%, cMET – 97.8%, P-	HGF SNP rs1800793 minor allele associated with increased risk of death. Stronger P-cMET correlated with decreased mortality (possibly more highly expressed in early stage disease).	[29]

					cMET – 95.3%.		
Circulating HGF and HGF/cMET expression	Blood samples from women with pelvic masses, and surgical resection material	Blood – 123, IHC - 81	ELISA and IHC – cellularity 0-4 scale	N/A	N/A	HGF >2 SD above reference value had s shorter disease-free survival, HGF higher in ovarian carcinoma patients, than those with benign or borderline tumours.	[31]
Ascites and ovarian cyst fluid.	Cyst fluid, ascites, and peritoneal fluid	52	ELISA	N/A	N/A	Median HGF levels significantly higher in ascites vs normal peritoneal fluid.	[32]
Met amplification	Archival FFPE samples	110	FISH	MET:CEP7 ratio >2	4%	MET amplification associated with a higher histological grade and development of more metastatic sites.	[54]
cMET over-expression and Met amplification	Clear cell ovarian carcinoma	90	FISH and IHC – intensity scale 0-3	FISH: MET:CEN7 ratio, and % of cells positive for MET>CEN7. IHC: 2-3 in	24% MET amplification, 22% by IHC.	cMET over-expression was identified as an independent unfavourable prognostic factor for overall survival.	[43]

				>10% of cells			
Met amplification and cMET over-expression	Clear cell ovarian carcinoma	73	FISH, qRT, and IHC	FISH: MET:CEP7 ratio >2. qRT: MET/hTERT ratio >1.5.	37% MET amplification.	Stage 1 and 2 patients with Met amplification had worse survival than patients without Met amplification.	[44]

Table 1 Publications addressing HGF/cMET biomarkers in ovarian cancer. IHC, immunohistochemistry. FISH, fluorescent in situ hybridisation. qRT, quantitative reverse transcriptase PCR. SNP, single nucleotide polymorphism. ELISA, enzyme-linked immunosorbent assay.

Agent	Other names	Company	Type	Target/s	In clinical trial/s (past and present)	Trials using biomarkers
ABT-700	h224G11	AbbVie	mAb	cMET	Yes, 1 listed	
AMG 208		Amgen	TKI	cMET, Ron	Yes, 2 listed	NCT00813384
AMG 337		Amgen	TKI	cMET	Yes, 4 listed	NCT02344810 NCT02016534 NCT02096666
ARGX-111		arGEN-x	mAb	cMET	Yes, 1 listed	NCT02055066
BMS-777607	ASLAN002	ASLAN pharmaceuticals, BMS	TKI	cMET, RON, AXL	Yes, 2 listed	
BMS-794833		BMS	TKI	cMET, AXL, FLT3, RON, VEGFR2	1, withdrawn.	
Cabozantinib	XL 184, BMS907351	Exelixis, BMS	TKI	cMET, VEGFR2, RET, KIT, FLT3, Tie2	Yes, 68 listed	NCT02008383 NCT01553656 NCT01639508
CGEN-241		Compugen	Decoy cMET		None listed.	
Crizotinib	PF02341066, Xalkori	Pfizer	TKI	cMET, ROS1, ALK, AXL, RON, Tie2	Yes, 88 listed	NCT02034981 NCT02499614 NCT01524926 NCT02435108 NCT02510001

E-7050	Golvantinib	Eisai	TKI	cMET, VEGFR2	Yes, 8 listed	
EMD 1204831		EMD Serono	TKI	cMET	1, terminated	
EMD-1214063	Tepotinib, MSC2156119J	EMD Serono	TKI	cMET	Yes, 1.	
Ficlatuzumab	AV-299, SCH900105	AVEO	mAb	HGF	Yes, 7 listed	NCT02277184 NCT02277197
Foretinib	XL 880, GSK1363089	Exelixis, GSK	TKI	cMET, VEGFR2, AXL, PDGFR, Kit, Tie2, FLT3	Yes, 11 listed	NCT00725764 NCT00725712 NCT01147484
INC280	INCB28060, Capmatinib	Incyte, Novartis	TKI	cMET	Yes, 21 listed	NCT01870726 NCT01737827 NCT02468661 NCT01964235 NCT02626234 NCT02520752 NCT01610336 NCT02276027 NCT02205398 NCT02323126 NCT02414139 NCT01324479 NCT01911507
JNJ-38877605		Johnson & Johnson	TKI	cMET	Yes, 1 listed	
LY-2801653	Merestinib	Eli Lilly	TKI	cMET, VEGFR2, RON, FLT3, AXL	Yes, 3 listed	

LY-2875358	Emibetuzumab	Eli Lilly	mAb	cMET	Yes, 6 listed	NCT01900652 NCT01874938
MGCD-265		Methylgene	TKI	cMET, RON, VEGFER1, VEGFR2, VEGFR3, Tie2.	Yes, 6 listed	NCT02544633 NCT00697632
MK 8033		Merck	TKI	cMET, RON	Yes, 1 listed	NCT00559182
MK-2461		Merck	TKI	cMET, RON, Flt 1/3, PDGFRβ	Yes, 2 listed	
MP470	Amuvatinib	SuperGen	TKI	cMET, cKit, PDGFR, FLT3, AXL	Yes, 4 listed	
MSC2156119J	EMD 1214063, Tepotinib	Merck, EMD Serono	TKI	cMET	Yes, 5 listed	NCT01832506 NCT01014936 NCT01982955 NCT01988493 NCT02115373
NK4		Kringle Pharma	HGF antagonist		None listed	
NVP-BVU972		Novartis	TKI	cMET	None listed	
Onartuzumab	OA5D5, MetMab, RO5490258	Genentech, Roche	mAb	cMET	Yes, 17 listed	NCT01974258 NCT01519804 NCT01632228 NCT02044601 NCT01590719 NCT01662869 NCT01887886 NCT02031744 NCT01496742

						NCT01456325 NCT00854308
PF-04217903		Pfizer	TKI	cMET, ALK	1, terminated.	
PHA-665752		Pfizer	TKI	cMET	None listed	
Rilotumumab	AMG 102	Amgen	mAb	HGF	Yes, 15 listed	NCT02137343 NCT01039207 NCT01697072 NCT00422019 NCT02154490
Sar125844		Sanofi	TKI	cMET	Yes, 3 listed	NCT01657214 NCT01391533 NCT02435121
SGX-523		SGX Pharmaceuticals	TKI	cMET	2, both terminated	
SU11274		Sugen	TKI	cMET	None listed	
TAK-701		Millennium	mAb	HGF	Yes, 1 listed	
Tas 115		Taiho	TKI	cMET, VEGFR	None listed	
Tivantinib	ARQ197	ArQule, Daiichi Sankyo	TKI, non-ATP competitive	cMET	Yes, 46 listed	NCT01447914 NCT01749384 NCT01395758 NCT01625156 NCT01725191 NCT01892527 NCT01696955 NCT01688973 NCT01244191 NCT01755767

						NCT01861301 NCT01575522 NCT00612209 NCT00777309 NCT00874042 NCT00827177 NCT00802555 NCT01468922 NCT02029157
Volitinib	HMPL-504, AZD6094, Savolitinib	Hutchison Medipharma Limited	TKI	cMET	Yes, 9 listed	NCT02252913 NCT02374645 NCT02449551 NCT01773018 NCT02447406 NCT02447380

Table 2. Agents targeting HGF/cMET. mAb, monoclonal antibody. TKI, tyrosine kinase inhibitor. BMS, Bristol-Myers Squibb. GSK, GlaxoSmithKline.

Agent	Clinical trials targeted at patients with ovarian cancer	Publications specifying results for patients with ovarian cance
AMG 208	-	[99]
Cabozantinib	NCT01716715 NCT02315430	Buckanovich et al, ASCO 2011, abstract 5008
Ficlatuzumab	NCT02090127, continued access	[61]
Onartuzumab	-	[100]
Rilotumumab	NCT01039207	[71, 88]
Tivantinib	-	[97, 98, 101]

Table 3. Agents targeting HGF/cMET in ovarian cancer-specific clinical and publications describing disease results.

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